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*[Continued on next page]*

(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF IMMUNE RELATED DISEASES

MKPTLCFLFILVSLFPLIVPGNAQCSFESLVDQRIKEALSRQEPKTISCTSVTSSGRLASCPAGMVVTG
CACGYGCGSWDIRNGNTCHCQCSVMDWASARCCRMA

Signal sequence

1-20

Transmembrane domain
none

N-myristoylation site.

21-26
64-69
69-74

Prenyl group binding site (CAAX box).

102-106

(57) Abstract: The present invention relates to compositions containing novel proteins and methods of using those compositions for the diagnosis and treatment of immune related diseases.

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alternatively at least about 90% amino acid sequence identity, alternatively at least about 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as herein before described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

Another aspect the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO polypeptide as defined herein. In a particular embodiment, the agonist or antagonist is an anti-PRO antibody or a small molecule.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprise contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native PRO polypeptide.

In a still further embodiment, the invention concerns a composition of matter comprising a PRO polypeptide, or an agonist or antagonist of a PRO polypeptide as herein described, or an anti-PRO antibody, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as herein before described, or an anti-PRO antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence (SEQ ID NO:1) of a native sequence PRO1081 cDNA, wherein SEQ ID NO:1 is a clone designated herein as "DNA54229-1366".

Figure 2 shows the amino acid sequence (SEQ ID NO:2) derived from the coding sequence of SEQ ID NO:1 shown in Figure 1.

Figure 3 shows a nucleotide sequence (SEQ ID NO:3) of a native sequence PRO1274 cDNA, wherein SEQ ID NO:3 is a clone designated herein as "DNA64889-1541".

Figure 4 shows the amino acid sequence (SEQ ID NO:4) derived from the coding sequence of SEQ ID NO:3 shown in Figure 3.

Figure 5 shows a nucleotide sequence (SEQ ID NO:5) of a native sequence PRO1199 cDNA, wherein SEQ ID NO:5 is a clone designated herein as "DNA65351-1366".

Figure 6 shows the amino acid sequence (SEQ ID NO:6) derived from the coding sequence of SEQ ID NO:5 shown in Figure 5.

Figure 7 shows a nucleotide sequence (SEQ ID NO:7) of a native sequence PRO1754 cDNA, wherein SEQ ID NO:7 is a clone designated herein as "DNA76385-1692".

Figure 8 shows the amino acid sequence (SEQ ID NO:8) derived from the coding sequence of SEQ ID NO:7 shown in Figure 7.

Figure 9 shows a nucleotide sequence (SEQ ID NO:9) of a native sequence PRO1556 cDNA, wherein SEQ ID NO:9 is a clone designated herein as "DNA76529-1666".

Figure 10 shows the amino acid sequence (SEQ ID NO:10) derived from the coding sequence of SEQ ID NO:9 shown in Figure 9.

Figure 11A-11B shows a nucleotide sequence (SEQ ID NO:11) of a native sequence PRO4401 cDNA, wherein SEQ ID NO:11 is a clone designated herein as "DNA84912-2610".

Figure 12 shows the amino acid sequence (SEQ ID NO:12) derived from the coding sequence of SEQ ID NO:11 shown in Figure 11A-11B.

Figure 13 shows a nucleotide sequence (SEQ ID NO:13) of a native sequence PRO9912 cDNA, wherein SEQ ID NO:13 is a clone designated herein as "DNA108700-2802".

Figure 14 shows the amino acid sequence (SEQ ID NO:14) derived from the coding sequence of SEQ ID NO:13 shown in Figure 13.

Figure 15 shows a nucleotide sequence (SEQ ID NO:15) of a native sequence PRO10268 cDNA, wherein SEQ ID NO:15 is a clone designated herein as "DNA145583-2820".

Figure 16 shows the amino acid sequence (SEQ ID NO:16) derived from the coding sequence of SEQ ID NO:16 shown in Figure 16.

Figure 17 shows a nucleotide sequence (SEQ ID NO:17) of a native sequence PRO10272 cDNA, wherein SEQ ID NO:17 is a clone designated herein as "DNA147531-2821".

Figure 18 shows the amino acid sequence (SEQ ID NO:18) derived from the coding sequence of SEQ ID NO:17 shown in Figure 17.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

### I. Definitions

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (i.e., PRO/number) refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided as an actual numerical designation as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. The term "PRO polypeptide" refers to each individual PRO/number polypeptide disclosed herein. All disclosures in this

and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington) if necessary.

Based on the consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence. Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probe sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5kbp. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, *supra*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

A pool of 50 different human cDNA libraries from various tissues was used in cloning. The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science, 253:1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for a full-length polypeptide

EXAMPLE 6: Isolation of cDNA Clones Encoding Human PRO Polypeptides

Using the techniques described in Examples 1 to 5 above, numerous full-length cDNA clones were identified as encoding PRO polypeptides as disclosed herein. These cDNAs were then deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC) as shown in Table 7 below.

Table 7

| Material | UNQ | PRO | ATCC # | ATCC Deposit Date |
|---|---|---|---|---|
| DNA54229-1366 | 538 | 1081 | 209803 | April 23, 1998 |
| DNA64889-1541 | 644 | 1274 | 203250 | September 9, 1998 |
| DNA65351-1366 | 612 | 1199 | 209856 | May 12, 1998 |
| DNA76385-1692 | 827 | 1754 | 203664 | February 9, 1999 |
| DNA76529-1666 | 764 | 1556 | 203315 | October 6, 1998 |
| DNA84912-2610 | 1926 | 4401 | 203964 | April 27, 1999 |
| DNA108700-2802 | 3077 | 9912 | PTA-1093 | December 22, 1999 |
| DNA145583-2820 | 3119 | 10268 | PTA-1179 | January 11, 2000 |
| DNA147531-2821 | 3120 | 10272 | PTA-1185 | January 11,2000 |

EXAMPLE 7:Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no.24)

This example shows that the polypeptides of the invention are active as stimulators of the proliferation of T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may also take the form of antagonists of the PRO polypeptides of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide, which would be expected to inhibit T-lymphocyte proliferation.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% $CO_2$ )and then washed and resuspended to 3 x $10^6$ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate).

The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads). The assay is prepared by plating in triplicate wells a mixture of: 100µl of test sample diluted to 1% or to 0.1%; 50 µl of irradiated stimulator cells and 50 µl of responder PBMC cells. 100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% $CO_2$ for 4 days. On day 5 and each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI;10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to 1x $10^7$ cells/ml of assay media. The assay is then conducted as described above. The results of this assay for compounds of the invention are shown below in Table 8. Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

Table 8

| PRO | PRO Concentration | Percent Increase Over Control |
|---|---|---|
| PRO1081 | 18.39 nM | 275.5 |
| PRO1274 | 58.32 nM | 230.1 |
| PRO10272 | 0.84 nM | 201.5 |

EXAMPLE 8: Skin Vascular Permeability Assay (no.64)

This assay shows that certain PRO polypeptides stimulate an immune response and induce inflammation by inducing mononuclear cell, eosinophil and PMN infiltration at the site of injection of the animal. This skin vascular permeability assay is conducted as follows. Hairless guinea pigs weighing 350 grams or more are anesthetized with ketamine (75-80 mg/Kg) and 5 mg/Kg Xylazine intramuscularly (IM). A sample of purified PRO polypeptide or a conditioned media test sample is injected intradermally onto the backs of the test animals with 100 µL per injection site. It is possible to have about 10-30, preferably about 16-24, injection sites per animal. One mL of Evans blue dye (1% in physiologic buffered saline) is injected intracardially. Blemishes at the injection sites are then measured (mm diameter) at 1hr, 6 hrs and 24 hrs post injection. Animals were sacrificed at the appropriate time after injection. Each skin injection site is biopsied and fixed in paraformaldehyde. The skins are then prepared for histopathalogic evaluation. Each site is evaluated for inflammatory cell infiltration into the skin. Sites with visible inflammatory cell inflammation are scored as positive. Inflammatory cells may be neutrophilic, eosinophilic, monocytic or lymphocytic

At least a minimal perivascular infiltrate at the injection site is scored as positive, no infiltrate at the site of injection is scored as negative. Results are given in Table 9.

Table 9

| PRO | Time (hrs) | Infiltrate |
|---|---|---|
| PRO1754 | 6.00 | positive |
| PRO9912 | 6.00 | positive |

EXAMPLE 9: Inhibitory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no. 67)

This example shows that one or more of the PRO polypeptides are active as inhibitors of the proliferation of stimulated T-lymphocytes. Compounds which inhibit proliferation of lymphocytes are useful therapeutically where suppression of an immune response is beneficial.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% $CO_2$) and then washed and resuspended to $3x10^6$ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:1 of test sample diluted to 1% or to 0.1%,

50 :1 of irradiated stimulator cells, and

50 :1 of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% $CO_2$ for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to $1x10^7$ cells/ml of assay media. The assay is then conducted as described above.

Any decreases below control is considered to be a positive result for an inhibitory compound, with decreases of less than or equal to 80% being preferred. However, any value less than control indicates an inhibitory effect for the test protein. Results are given in Table 10.

Table 10

| PRO | PRO Concentration | Percent Decrease Below Control |
|---|---|---|
| PRO1199 | 4.97 nM | 70 |
| PRO1199 | 49.65 nM | 66.8 |
| PRO1199 | 119.99 nM | 56.6 |
| PRO1556 | 2.75 nM | 66.2 |
| PRO4401 | 1.42 nM | 73.6 |
| PRO4401 | 14.16 nM | 66.3 |
| PRO10268 | 1.77 nM | 75.0 |
| PRO10268 | 17.66 nM | 18.0 |

EXAMPLE 10: *In situ* Hybridization

*In situ* hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis and aid in chromosome mapping.

*In situ* hybridization was performed following an optimized version of the protocol by Lu and Gillett, *Cell Vision* 1: 169-176 (1994), using PCR-generated $^{33}P$-labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinated in proteinase K (20 mg/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A [$^{33}P$] UTP-labeled antisense riboprobe was generated from a PCR product and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2 nuclear track emulsion and exposed for 4 weeks.

# FIGURE 6

MKALCLLLLPVLGLLVSSKTLCSMEEAINERIQEVAGSLIFRAISSIGLECQSVTSRGD
LATCPRGFAVTGCTCGSACGSWDVRAETTCHCQCAGMDWTGARCCRVQP

Signal peptide:
1-18

Transmembrane domain:
none

Cell attachment sequence.
57-60

N-myristoylation site.

13-19
71-77
75-81
95-101
100-106